Received Date : 10-Oct-2019
Revised Date : 17-Jan-2020
Accepted Date : 03-Feb-2020
Article type : State-of-the-Art Review

NKT CELLS AND THE REGULATION OF INTESTINAL IMMUNITY: A TWO-WAY STREET

Phillip M. Brailey[1,2,*], Marta Lebrusant-Fernandez[1,2,*] & Patricia Barral[1,2]

[1]The Peter Gorer Department of Immunobiology. King's College London. London, UK
[2]The Francis Crick Institute. London, UK
*Equal contribution

Corresponding author: Patricia Barral
Tel: +44 (0)2037963358
E-mail: patricia.barral@kcl.ac.uk

Running title: The crosstalk between the microbiota and NKT cells

Key words: NKT cells, CD1, microbiota, lipid antigens, IBD

ABBREVIATIONS

αGalCer: alpha-galactosylceramide

APC: antigen presenting cells

CD: Crohn's disease

DC: dendritic cell

DSS: dextran sodium sulfate

GF: germ-free

IBD: Inflammatory Bowel Disease

IEC: intestinal epithelial cells

ILC: Innate lymphoid cell

mLN: mesenteric lymph node

NKT cell: Natural Killer T cell

PLZF: promyelocytic leukemia zinc finger

RORγt: RAR-related orphan receptor gamma

SI: small intestine

SPF: specific pathogen free

TCR: T cell receptor

TLR: Toll like receptor

UC: ulcerative colitis

ABSTRACT

The mammalian gastrointestinal compartment is colonised by millions of microorganisms that have a central influence on human health. Intestinal homeostasis requires a continuous dialogue between the commensal bacteria and intestinal immune cells. While interactions between host and commensal bacteria are normally beneficial, allowing training and functional tuning of immune cells, dysregulated immune system-microbiota crosstalk can favour the development of chronic inflammatory diseases, as it is the case for inflammatory bowel disease (IBD). Natural Killer T (NKT) cells, which recognize CD1-restricted microbial and self-lipids, contribute to the regulation of mucosal immunity by controlling intestinal homeostasis and participating in the development of IBD. Here, we provide an overview of the recently identified pathways underlying the crosstalk between commensal bacteria and NKT cells and discuss the effect of these interactions in intestinal health and disease.

INTRODUCTION

The mammalian gastrointestinal mucosa is a unique environment colonised by a highly complex mixture of microorganisms that establish a mutualistic relationship with the host. The defence system of the intestinal mucosa comprises an epithelial layer and a plethora of immune cells that restrict commensals within the intestine while preserving their number and diversity. Conversely, commensal organisms are required for the development of a fully functional immune system [1]. While interactions between host and commensal bacteria are normally beneficial, it is becoming clear that dysregulated immune system-microbiota crosstalk can favour the development of chronic inflammatory diseases such as inflammatory bowel disease (IBD). Metagenomic sequencing studies reveal alterations in the populations of commensal bacteria (dysbiosis) in patients suffering from IBD. Moreover, studies in murine models provide a causal link between changes in commensal populations and the pathogenesis and progression of intestinal inflammation [2]. The factors triggering the development of IBD are incompletely understood, but the balance between health and disease is most likely regulated by the interactions between immune cells and commensal microbes. Hence, deciphering the mechanisms that mediate the communications between microbial communities and immune cells could provide approaches to prevent or ameliorate intestinal disorders, with potential relevance to immunopathologies more generally.

Whilst much gut immunology research has focused on the function of conventional MHC-restricted T cells, recent studies have revealed a central role for CD1-restricted, lipid-reactive Natural Killer T (NKT) cells in the regulation of intestinal immunity. NKT cells are present in mouse and human gut where they recognise commensal-derived lipids and participate in the control of intestinal homeostasis and inflammation. In this review we will summarise the mechanisms underlying the crosstalk between the commensal microbiota and NKT cells and discuss how these interactions shape mucosal immunity in health and disease.

NKT CELLS: AN OVERVIEW

The mammalian immune system has evolved to protect hosts from a vast variety of threats through the evolutionarily “ancient” innate immunity and the “modern” adaptive immunity. NKT cells fall at the crossroads of these two immune paradigms, capable of recognising antigens through their T cell receptor (TCR), but responding rapidly in an innate-like manner. These abilities warrant NKT cells a place within the “unconventional T cell” family, along with MAIT cells and $\gamma\delta$ T cells [3, 4].

NKT cell development and classification

NKT cells were originally identified by the expression of NK markers as well as their TCR, and later defined for their capacity to recognise lipid antigens presented by the MHC-I-like molecule CD1 [3, 5]. CD1 molecules encompass a family of lipid-presenting proteins (CD1a-d in humans, with only CD1d expressed in mice) that vary in the structure of their lipid binding grooves, patterns of cellular expression and intracellular trafficking [3, 6]. Within the thymus $CD1^+CD4^+CD8^+$ thymothytes participate in the selection of NKT cells that will consequently become CD1 restricted [7]. While CD1 is strictly required for NKT cell development, the nature of the lipid ligands involved in NKT cell selection remains unclear and controversial [8, 9]. Based on their CD1 restriction and TCR specificity NKT cells can be classified in two families [4]: type I or type II NKT cells. Type I NKT cells - also known as invariant NKT (iNKT) cells - express a semi-invariant TCR (typically Vα14-Jα18 in mice and Vα24-Jα18 in humans) and recognise the lipid α-galactosylceramide (αGalCer) presented by CD1d. These cells can be identified using αGalCer-loaded CD1d tetramers, and they have been the most studied group up to date. Despite their limited TCRα usage, iNKT cells recognise diverse endogenous and exogenous lipid antigens. Recognition of diverse lipids is partially dependent on the TCRVβ usage but also on variations of the polymorphic CDR3β region [10-12]. On the other hand, type II NKT cells are not reactive to αGalCer and recognise a variety of lipids presented by the different CD1 molecules (CD1a-d) [13]. A proportion of type II NKT cells recognises the naturally occurring self-glycolipid sulfatide and has an oligoclonal TCR repertoire [14]. However, sulfatide-loaded CD1d tetramers have not been widely used to characterize type II NKT cells due to high background staining. As they lack a defining marker, the function of type II NKT cells in mice is usually deduced by comparing the phenotype of CD1d-deficient mice (which lack type I and II NKT cells) with Jα18-deficient mice (which lack type I NKT cells).

iNKT cell functional subsets

iNKT cells show a wide tissue distribution but, unlike conventional T cells, the majority of iNKT cells don't recirculate but instead establish long-term residency within the tissues [15-18]. In peripheral tissues of mice, we can find several functional subsets of iNKT cells that are classified on the basis of their transcription factor expression and their cytokine secretion patterns (Table 1): NKT1 ($T\text{-}bet^+$), NKT2 ($PLZF^{hi}$), NKT17 ($ROR\gamma t^+$), NKT10 ($E4BP4^+$), NKTreg ($Foxp3^+$) and NKT follicular helper cells (NKT_{FH}, $Bcl6^+$). NKT1, NKT2 and NKT17 are found at different proportions in various lymphoid and non-lymphoid tissues in homeostatic conditions [19-21]. In C57Bl/6 mice NKT1 cells are highly abundant and especially enriched in liver, spleen and thymus, NKT2 cells

are abundant in mesenteric lymph node (mLN), and NKT17 cells are enriched in lungs as well as in inguinal, axillary and cervical LNs [20]. NKT10 preferentially accumulate in adipose tissue in homeostasis, but can be induced in response to immunisation and are found in the intestine of transgenic mouse models of colorectal cancer ($APC^{Min/+}$ mice) [18, 22, 23]. On the other hand, NKT_{FH} and NKTregs have been only detected in mice during immune responses [24, 25]. In agreement with their distinct phenotype, iNKT cells found in various tissues have unique functions that contribute to modulate the outcome of immunity [16]. For instance, in the lung-draining LNs iNKT cells are major producers of IL-4 and defend against viral infection [26], while adipose tissue iNKT cells secrete IL-10 and protect from inflammation-induced obesity [18].

While the preferential tissue distribution of the various iNKT cell subsets is well stablished, the mechanisms controlling their differentiation in the thymus and/or the periphery remain incompletely understood. Several (but non-exclusive) scenarios could contribute to explain the differential presence of iNKT cell subsets in various anatomical locations: (i) iNKT cells commit in the thymus before migrating to peripheral tissues; (ii) uncommitted iNKT cells emigrate from the thymus and differentiate in peripheral tissues; (iii) functionally committed iNKT cells display plasticity in response to specific signals and/or (iv) certain iNKT cell subsets can preferentially expand in response to signals in the tissues or during an immune response. A broad range of literature backs these various scenarios. For instance, in support of thymic commitment it has been shown that the TCR signal strength during thymic development can direct the differentiation of iNKT cells within specific functional subsets [27]. Further to this, a variety of signalling molecules and transcription factors including Zap70, Bcl11b or Roquin have been shown to participate in the control of iNKT cell lineage differentiation [28-30]. On the other hand, several lines of evidence suggest that iNKT cells can acquire functional capabilities in the periphery. For instance, earlier studies showed that non-committed iNKT cells can be induced to produce IL-9 or IL-17 in response to cytokine stimulation [31, 32]. Also, it has been recently shown that undifferentiated $PLZF^{hi}CCR7^{+}$ iNKT cell precursors can emigrate from the thymus to the periphery where they terminally differentiate into one of the iNKT cell subsets [33]. These iNKT cell precursors are found in various peripheral tissues and show a phenotype and TCR repertoire distinct from that of mature iNKT cells [12, 33, 34]. Finally, specific iNKT cell subsets can expand in various tissues in response to lipid antigen administration. For instance, NKT2 cells expand in the mLN in response to oral lipids, while pre-treatment with αGalCer induces the expansion of NKT10 cells in the spleen [22, 35]. Thus, all together this data suggests that while thymic signals modulate the commitment of iNKT cells to one of the functional subsets it is likely that peripheral signals may also contribute to shape the phenotype and function of iNKT cells in specific tissues.

iNKT cell activation

NKT cells can get activated through antigen-dependent and independent mechanisms and in response to foreign or self-lipids (Figure 1). CD1d-mediated presentation of exogenous lipid antigens to iNKT cells leads to their activation and rapid cytokine production [36]. For instance, iNKT cells can recognise and be activated by CD1d-restricted foreign lipids either synthetic or found in pathogenic bacteria (e.g. *Streptococcus pneumonia*, *Borrelia Burgdorgferi*), commensals (*Bacteroides fragilis*), fungi (*Aspergillus fumigatus*) or allergenic sources (pollens) [37-40]. Co-stimulatory molecules can positively (e.g. ICOS, CD40L, CD28) or negatively (e.g. PD-1, BTLA, LAG-3) influence iNKT cell activation and skew the immune response [41]. In the absence of exogenous lipids, iNKT cells can also be activated by self-lipids in a variety of contexts [42]. For instance, in the presence of innate-like signals (e.g. during viral infection) activation of iNKT cells can still occur in a CD1d-dependent manner. In this case, Toll like receptor (TLR) stimulation of antigen presenting cells (APCs) induces changes in lipid biosynthesis and accumulation of endogenous lipids which are presented on CD1d and mediate iNKT cell activation [8, 43-45]. Lastly, cytokines can further enhance TCR-dependent responses and, in some instances, iNKT cells can be activated solely in response to cytokine stimulation (e.g. IL-12, IL-18) [36, 46, 47]. Importantly, these mechanisms of activation are not mutually exclusive. In some instances, despite the presence of CD1d-restricted lipid antigens, cytokine-driven signals - rather than cognate antigens - dictate iNKT cell activation as it has been shown during infections with *Sphingomonas yanoikuyae* or *Streptococcus pneumoniae* [48].

NKT CELLS IN THE INTESTINAL COMPARTMENT

The gastrointestinal tract constitutes one of the most complex antigenic environments in the body as it is not only continually exposed to dietary products but it is also densely populated with a vast array of intestinal bacteria. As a result, the intestine is equipped with a highly specialised immune system that is key for maintaining the segregation between the host and bacteria consequently controlling tissue homeostasis [1]. Containment of commensals within the intestine is achieved by an epithelial layer together with populations of B cells, T cells, macrophages, dendritic cells (DCs) and various innate immune cells including unconventional T cells.

NKT cells are found in the intestine of mice and humans. In humans, iNKT cells are present at high frequencies in the foetal intestine while in mice intestinal iNKT cells are absent at birth but their frequency increases over the first 6 weeks of age [49-51]. In the gut of adult mice iNKT cells account for up to 5% of TCRβ^+ T cells, with varying frequencies across the gut mucosa [35, 52].

iNKT cell numbers inversely correlate with both bacterial density and bacterial proximity, and thus iNKT cells are more prevalent in the small intestine (SI) than in the colon and more highly represented in the lamina propria than in the epithelial layer [35, 52]. As it is the case in most peripheral tissues, NKT1 cells are prevalent in the intestinal tract representing around ~85% of iNKT cells in the SI, colon and Peyer's Patches of C57BL/6 mice, with the rest of the cells belonging to NKT17 (~10%) and NKT2 (<5%) families [35, 53]. NKT1 cells are also prevalent in the gut-draining mesenteric lymph nodes (mLN), although there is an increased presence of NKT2s (~40%) in this tissue. Consistent with this, NKT cells are the major producers of IL-4 in this tissue and after stimulation of mLN lymphocytes with PMA/ionomycin iNKT cells constitute up to 60% of the IL-4 producing cells [35]. Finally, NKT10 cells are also found in the intestine of transgenic mouse models of colorectal cancer ($APC^{Min/+}$ mice) [23]. It is worth noting that while the majority of iNKT cells found in the adult murine gut in steady-state are T-bet$^+$ NKT1s, it is possible that other iNKT cell subsets (or precursors) could be recruited/expanded under certain inflammatory conditions ultimately shaping the functional capabilities of the local iNKT cell population.

Regulation of NKT cells' homeostasis by the microbiota

It is increasingly appreciated that commensal-derived products regulate the homeostasis and function of most immune cell populations. Commensal bacteria represent a major source of lipids, several of which have been identified for their capacity to activate iNKT cells. Indeed, cumulative evidence demonstrates that the microbiota provides key signals for modulating intestinal iNKT cell numbers and phenotype, although iNKT cells are present in the intestine even in the absence of microbiota [50, 52, 54]. In homeostatic conditions iNKT cells from the gut and mLN (but not from spleen or liver) express high levels of the transcription factor Nur77 (as detected in reporter mice), suggesting that intestinal iNKT cells are recognising lipid antigens in a TCR-dependent manner [35, 55]. The influence of microbial-derived products in the control of the iNKT cell population was confirmed in experiments with germ-free (GF) mice, where iNKT cells are present at higher numbers in the colon and exhibit a less mature phenotype in comparison with cells from Specific Pathogen free (SPF) mice [50, 52]. The effect of the microbiota on the iNKT cell population seems to be particularly important early in life as iNKT cells proliferate and accumulate in the intestine of neonatal GF mice in a process that is negatively regulated by exposure to commensals [50, 51]. Not only the presence/absence but also the composition of the commensal bacteria can have profound effects on iNKT cells. For instance, short-term antibiotic treatment is sufficient to induce the expansion of colonic iNKT cells and to alter their cytokine profile [56]. Moreover, the presence of dysbiotic microbiota after antibiotic treatment generates a

proinflammatory phenotype on colonic iNKT cells [56]. Strikingly, the gut microbiome is also able to regulate iNKT cells in distal tissues including the spleen, liver and thymus [52, 54]. iNKT cells in these tissues of mice from different vendors with known variation in their intestinal bacteria (Taconic vs. Jacksons), differ in their frequency, phenotype, TCRVβ usage and cytokine secretion while these differences disappear after co-housing of the mice [52]. Moreover, the liver is exposed to gut-derived products through the portal vein and the microbiome can use bacterial bile acid metabolites as messengers to cause the accumulation of hepatic CXCR6-expressing iNKT cells. As a result, these cells present a more activated phenotype and mediate the inhibition of hepatic tumour growth [57].

Although the microbial-derived lipids that might be involved in the regulation and maintenance of the iNKT cell population in the gut remain largely uncharacterised, several key bacteria have been identified in recent years. *Sphingomonas*, which are commensal species in the gut, contain glycosylceramides on the cell wall that can serve as antigens for iNKT cells [8, 58, 59]. Indeed, iNKT cells from GF mice show an immature phenotype which is rescued after colonisation with *Sphingomonas* but not with *E. coli* (which lack glycosylceramides) [52]. Members of the *Bacteroides* genus may be crucial for regulating the iNKT cell population as 40-70% of their membrane phospholipids are sphingolipids, which could be presented to iNKT cells by CD1d. Indeed, an isoform of αGalCer is produced by *B. fragilis*. This sphingolipid was shown to activate and elicit IFN-γ production both in mouse and human iNKT lymphocytes in a CD1d-depedent manner [38]. Conversely, *B. fragilis* has been reported to have a direct effect on the homeostasis of colonic iNKT cells by providing inhibitory sphingolipids that negatively modulate cell proliferation in the neonatal period [51]. Interestingly, the presence of αGalCers in the murine intestinal compartment is strongly decreased in response to Western type diet, DSS-induced colitis or Influenza A virus infection, potentially impacting the function of intestinal iNKT cells [60]. Altogether these studies support a model by which the commensal microbiota can control the iNKT cell compartment by providing either inhibitory or stimulatory lipid antigens. Nonetheless it is likely that other signals in the intestinal environment such as cytokines, neurotransmitters or dietary components also contribute to the control of the intestinal iNKT cell population, although the nature and the mechanism of action of such signals remain unknown.

Besides microbial-derived antigens, key players in the modulation of intestinal immunity are the various populations of antigen presenting cells (APC) found in the intestinal compartment, which can differentially control tolerogenic and inflammatory responses. Indeed, the context of lipid presentation provided by various $CD1d^+$ APCs can shape the outcome of immune responses, by

controlling the activation, proliferation and the pattern of cytokine secretion of iNKT cells [61]. CD1d is expressed by numerous cells in the gut including B cells, dendritic cells (DC), macrophages, intestinal epithelial cells (IECs) and innate lymphoid cells (ILCs), all of which can efficiently internalise lipid antigens and present them to iNKT cells. Direct evidence regarding the role of lipid presentation by APCs in the control of intestinal iNKT cells comes from conditional CD1d-KO mice. Experiments with these mice demonstrate that CD1d expression in $CD11c^+$ cells (including DCs and macrophages) controls the phenotype of intestinal iNKT cells as mice with a conditional deletion of CD1d on $CD11c^+$ cells have a decrease in the frequency of NKT17 cells in the SI and mLN [35]. Moreover, $CD11c^+$ cells also control the activation of intestinal iNKT cells after oral administration of αGalCer [35]. While the function of other $CD1d^+$ cells in the regulation of intestinal iNKT cell homeostasis remains unexplored, it is likely that various cell types collaborate to positively or negatively control iNKT cell immunity in the gut. For instance, group 3 ILCs (ILC3) have been shown to mediate the peripheral selection of $CD4^+$ T cells by inducing MHC-mediated cell death of activated commensal bacteria-specific T cells [62, 63]. Since ILC3s express CD1d and are able to present lipid antigens [64], it is tempting to speculate that they could also participate in the control of the intestinal iNKT cell population by CD1-mediated presentation of commensal-derived lipids.

NKT cells in the maintenance of intestinal homeostasis

iNKT cells play an important role in the host enteric defence and maintenance of barrier function in the intestine both by controlling microbial colonisation and by orchestrating the function of other intestinal cells (Figure 2). There are accumulating lines of evidence that support a bidirectional interaction between gut iNKT cells and the intestinal microbiota and consequently iNKT lymphocytes can also influence the composition of the commensal bacterial populations. In fact, CD1d-KO mice have a different microbiota than WT littermates and intragastric administration of various bacterial species (including *Pseudomonas aeruginosa* or *E. coli*) results in increased colonisation of the SI in CD1d-KO mice compared with WT mice [35, 65]. Interestingly, the changes in commensal microbiota detected in CD1d-KO mice are recapitulated in mice with a conditional deletion of CD1d on $CD11c^+$ cells, suggesting that the crosstalk between NKT cells and DCs/macrophages controls the intestinal microbial populations [35].

Several mechanisms are in place in the intestinal compartment to minimise the direct contact between intestinal bacteria and the epithelial cell surface. These include the presence of a mucus layer secreted by Goblet cells and the production of anti-microbial peptides by IEC. iNKT cells and CD1d expression have also been shown to be required for maintaining the physical

segregation between IECs and the microbiota as CD1d-KO mice show altered bacterial stratification, with commensal bacteria found in close proximity to IEC [35, 65]. While the mechanisms controlling this phenotype haven't been fully elucidated it has been suggested that iNKT cells could modulate Paneth cell degranulation (and consequently anti-microbial peptide secretion) through production of IFN-γ [66]. Accordingly, CD1d-KO mice present a defect in Paneth cell's granule ultrastructure and ability to degranulate after bacterial colonisation [65].

Besides IECs, intestinal iNKT cells can directly (through CD1d-mediated interactions) or indirectly control the function of other intestinal immune cells with potential consequences for intestinal homeostasis. For instance, it is well established that NKT cells can control antibody production by B cells [24, 67-69]. In the intestine, IgA is the most abundant antibody contributing to bacterial stratification, and together with IgG they cooperate to modulate intestinal immunity [70, 71]. A population of tissue-resident iNKT cells has been recently described in the Peyer's patches and seem to critically control B cell responses [53]. As such, iNKT cells produce the majority of the IL-4 in the Peyer's patches and provide indirect help for B cell class switch to IgG1. As a result, NKT cell-deficient mice show decreased levels of IgG1 in faeces [53]. Alterations in the IgA repertoire have been also observed in CD1d-deficient mice, although these are a consequence of the altered microbiota present in this strain [35]. On the other hand, iNKT cells indirectly modulate the intestinal Treg population, which is known to play a critical role in the regulation of intestinal homeostasis [72]. As such, Treg numbers and frequencies are altered in the mLN and SI of CD1d-KO mice vs. littermate controls, while activation of iNKT cells in response to oral αGalCer results in increased numbers of peripherally induced Tregs [35]. Moreover, a population of regulatory iNKT cells in the intestine has been shown to locally promote intestinal polyp formation by enhancing Treg cell frequency in a mouse model of colorectal cancer [23]. Finally, CD1d-mediated NKT cell-APC interactions can also modulate APC activation and function and ultimately shape intestinal immunity. For instance, engagement of CD1d on ILC3s induces secretion of IL-22 [64], which in turn supports epithelial barrier and promotes barrier defence mechanisms against bacterial pathogens [73]. Also, ligation of CD1d on IEC leads to production of IL-10 which protects from intestinal inflammation [74]. All together these studies propose NKT cells/CD1 as central players in the maintenance of intestinal homeostasis by modulating the composition and stratification of the intestinal commensal bacteria as well as the function of immune and epithelial cells.

NKT cells in intestinal inflammation

Given their importance in gut homeostasis, the role of NKT cells during intestinal disease is under increased investigation. In fact, NKT cells have been identified as major players in intestinal inflammation in mice and humans. IBD represents a group of intestinal disorders that cause prolonged inflammation of the digestive tract. The aetiology of IBD is unclear, with genetics, environmental/lifestyle factors and microbiota associated with increased risk. IBD can be classified in two major forms of disease -ulcerative colitis (UC) and Crohn's disease (CD)- which differ in the extent of pathology and in their immune profile. In general terms immune responses are thought to be predominantly Th1 for CD and Th2 for UC [75], although it is worth noting that this is a somewhat basic classification which may not fully cover the complexity of the immune responses in human IBD. Dysregulation of CD1 expression and NKT cell activation are associated with intestinal inflammation in mice and humans and suggest a complex role for these cells in IBD. In general, NKT cells seem to be pathogenic in Th2-like intestinal inflammation, but protective in Th1 models [76, 77].

Intestinal NKT cells contribute to the fuelling of intestinal inflammation in UC through a mechanism mediated by IL-13 (Figure 3). In the oxazolone model of murine UC, iNKT cells accumulate in the gut, whereas there is striking protection from disease in CD1d-KO, Jα18-KO and IL-13-KO mice as well as in WT mice pre-treated with anti-CD1d or anti-IL-13 blocking antibodies [50, 76, 78]. In this model, IL-13 secretion seems to be regulated by IL-25, as blocking IL-25 signalling results in decreased IL-13 levels and improved gut inflammation [79]. On the same line, GF mice -which have increased numbers of iNKT cells in the gut- show increased pathology in response to oxazolone-induced colitis, with significantly higher IL-13 secretion [50, 51]. In humans, type II NKT cells are enriched in the mucosa from patients with UC and secrete IL-13 [80, 81]. The mechanisms of action for IL-13 in intestinal inflammation are not fully understood, but experiments with colonic cell line monolayers suggest that IL-13 can induce apoptosis of IECs as well as alteration of tight junction protein composition ultimately disturbing the intestinal barrier function [82, 83]. In the clinic, administration of monoclonal antibodies against IL-13 (tralokinumab) to UC patients did not significantly increase the proportion of patients who achieved clinical response (defined as a >3 point reduction in Mayo score), however it did increase the number of patients in clinical remission (defined as a total Mayo score of <2), suggesting that targeting IL-13 may be beneficial in a subset of UC patients [84, 85].

While the role of NKT cells and IL-13 in the control of Th2-models of colitis is well established, the identity of the main IL-13 producers in UC remains unclear. In models of oxazolone-colitis direct evidence for IL-13 secretion by intestinal iNKT cells is scarce and IL-13 production has been only

shown in a small proportion of mLN iNKT cells (~10%) and in ILC2s [79] while no data is available for colonic iNKT cells. Moreover, the vast majority of iNKT cells in SI and colon are NKT1 cells with only very small proportions (<5%) of NKT2 cells (which are reported to be the main producers of type 2 cytokines) [19, 35]. Intriguingly, in IL-13 reporter mice iNKT cells are poor producers of IL-13 even in settings of type 2 inflammation [86]. This effect was attributed to the reduced GATA-3 expression found in iNKT cells which drives production of IL-4 over IL-13 [86]. Thus, whilst iNKT cells are critical players in oxazolone-colitis, these observations question whether they are the main producers of IL-13. This raises the possibility that iNKT cells could function by indirectly modulating the secretion of IL-13 by other cell types such as ILC2s or conventional $CD4^+$ T cells. Alternatively, it is also possible that the intestinal inflammatory environment may induce a degree of functional plasticity on intestinal iNKT cells and/or drive the expansion/recruitment of iNKT cell precursors or of a population of IL-13 secreting iNKT cells that hasn't been detected to date. Finally, IL-13 could be secreted by type II NKT cells in mice (as described in human UC patients [80]), although this scenario wouldn't explain the protective phenotype of Jα18-KO mice in oxazolone-colitis [76].

Besides IL-13-mediated intestinal inflammation, NKT cells and CD1 have been also shown to control intestinal immunity through other mechanisms of action. For instance, CD1d-dependent regulation of IEC function can on itself control gut inflammation. In oxazolone-colitis IECs produce anti-inflammatory IL-10 in a CD1d-depedent manner critically controlling intestinal pathology [74]. Consequently, the deletion of CD1d in the intestinal epithelium increases oxazolone-induced inflammation, while non-epithelial cells contribute to pathogenic NKT cell activation [74]. Interestingly, oxazolone compounds can be found in microbial and industrial sources and can activate iNKT cells and trigger CD1d-dependent intestinal inflammatory responses [87]. On a different experimental setting, overexpression of CD1d in a transgenic mouse model for type II iNKT cells is sufficient to induce the spontaneous development of intestinal inflammation; suggesting that interaction of NKT cells with CD1d-expressing cells can lead to the dysregulation of type II NKT cell responses and subsequently contribute to the development of IBD [88].

While the key role of NKT cells in Th2-driven models of colitis -such as oxazolone- is well established, their functions in CD and CD-like mouse models remain controversial. In humans, iNKT cells from the lamina propria of CD patients produce pro-inflammatory TNFα, IFNγ, IL-17A and IL-13 and can disrupt epithelial monolayer integrity, suggesting that they could contribute to disease pathology [89]. In the dextran sodium sulphate (DSS) model of IBD, NKTs have been shown to be protective in some studies but have no effect in some others. Original studies

showed that CD1d-deficient mice developed similar pathology to control mice after DSS administration [90]. However, other studies found that iNKT cells protect against DSS induced colitis by secreting IL-9 or by suppressing pathogenic T cells [77, 91]. While the reason for these discrepancies are not completely clear, they could be related to alterations in the microbiota in the NKT cell-deficient strains. It has been shown that CD1d-KO mice harbour an altered intestinal microbiota that is associated with exacerbated intestinal inflammation at steady-state and following DSS treatment [92]. This altered microbiota is transmissible upon faecal transplant to WT mice and influences iNKT cell function during DSS colitis [92]. Strikingly, other authors have described a less inflammatory microbiota that aids anti-inflammatory neutrophil recruitment in iNKT cell-deficient mice [93]. Thus, alterations in the microbiota will have a critical influence on the outcome of colitis and will likely contribute to the disparate functions proposed for NKT cells in this disease.

CONCLUSIONS AND FUTURE DIRECTIONS

An increasing number of studies reveal NKT cells as important modulators of homeostasis and inflammation in the gastrointestinal tract. NKT cells are able to control the composition of the intestinal microbiota and to modulate the function of other immune cells ultimately regulating intestinal homeostasis and the progression of inflammatory diseases. On the other hand, within the intestine NKT lymphocytes are exposed to a vast array of microbial-derived glycolipids, which markedly shape their phenotype and functions. It is thus obvious that there is a bidirectional communication between NKT cells and the intestinal environment that contributes to the control of gut immunity. Despite these advances, many questions remain open regarding the mechanisms controlling this crosstalk and the effect that they exert on mucosal immunity. For instance: Do dietary lipids, cytokines or neurotransmitters contribute to the control of intestinal NKT cell function? Is the antigen specificity of intestinal NKT cells regulated by commensals and/or altered in IBD? What are the mechanisms controlling NKT cell activation and effector functions in IBD? These are critical questions whose answers will provide a great leap forward in our understanding of host-microbiota interactions and will offer rationale for the design of new therapies to prevent or ameliorate intestinal disorders.

REFERENCES

1 Hooper LV, Littman, DR & Macpherson, AJ (2012) Interactions Between the Microbiota and the Immune System. *Science* **336**, 1268-1273.

2 Belkaid Y & Hand, TW (2014) Role of the microbiota in immunity and inflammation. *Cell* **157**, 121-41.

3 Mori L, Lepore, M & De Libero, G (2016) The Immunology of CD1- and MR1-Restricted T Cells. *Annu Rev Immunol* **34**, 479-510.

4 Godfrey DI, Uldrich, AP, McCluskey, J, Rossjohn, J & Moody, DB (2015) The burgeoning family of unconventional T cells. *Nat Immunol* **16**, 1114-23.

5 Godfrey DI, MacDonald, HR, Kronenberg, M, Smyth, MJ & Van Kaer, L (2004) NKT cells: what's in a name? *Nat Rev Immunol* **4**, 231-7.

6 Moody DB & Cotton, RN (2017) Four pathways of CD1 antigen presentation to T cells. *Curr Opin Immunol* **46**, 127-133.

7 Verykokakis M & Kee, BL (2018) Transcriptional and epigenetic regulation of innate-like T lymphocyte development. *Curr Opin Immunol* **51**, 39-45.

8 Mattner J, Debord, KL, Ismail, N, Goff, RD, Cantu, C, Zhou, D, Saint-Mezard, P, Wang, V, Gao, Y, Yin, N, Hoebe, K, Schneewind, O, Walker, D, Beutler, B, Teyton, L, Savage, PB & Bendelac, A (2005) Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* **434**, 525-9.

9 Porubsky S, Speak, AO, Luckow, B, Cerundolo, V, Platt, FM & Grone, HJ (2007) Normal development and function of invariant natural killer T cells in mice with isoglobotrihexosylceramide (iGb3) deficiency. *PNAS* **104**, 5977-82.

10 Mallevaey T, Scott-Browne, JP, Matsuda, JL, Young, MH, Pellicci, DG, Patel, O, Thakur, M, Kjer-Nielsen, L, Richardson, SK, Cerundolo, V, Howell, AR, McCluskey, J, Godfrey, DI, Rossjohn, J, Marrack, P & Gapin, L (2009) T cell receptor CDR2 beta and CDR3 beta loops collaborate functionally to shape the iNKT cell repertoire. *Immunity* **31**, 60-71.

11 Cameron G, Pellicci, DG, Uldrich, AP, Besra, GS, Illarionov, P, Williams, SJ, La Gruta, NL, Rossjohn, J & Godfrey, DI (2015) Antigen Specificity of Type I NKT Cells Is Governed by TCR beta-Chain Diversity. *J Immunol* **195**, 4604-14.

12 Jimeno R, Lebrusant-Fernandez, M, Margreitter, C, Lucas, B, Veerapen, N, Besra, GS, Fraternali, F, Spencer, J, Anderson, G & Barral, P (2019) Tissue-specific shaping of the TCR repertoire and antigen specificity of iNKT cells. *Elife* **8**.

13 Marrero I, Ware, R & Kumar, V (2015) Type II NKT Cells in Inflammation, Autoimmunity, Microbial Immunity, and Cancer. *Frontiers in immunology* **6**, 316.

14 Jahng A, Maricic, I, Aguilera, C, Cardell, S, Halder, RC & Kumar, V (2004) Prevention of autoimmunity by targeting a distinct, noninvariant CD1d-reactive T cell population reactive to sulfatide. *J Exp Med* **199**, 947-57.

15 Fan X & Rudensky, AY (2016) Hallmarks of Tissue-Resident Lymphocytes. *Cell* **164**, 1198-211.

16 Crosby CM & Kronenberg, M (2018) Tissue-specific functions of invariant natural killer T cells. *Nat Rev Immunol* **18**, 559-574.

17 Thomas SY, Scanlon, ST, Griewank, KG, Constantinides, MG, Savage, AK, Barr, KA, Meng, F, Luster, AD & Bendelac, A (2011) PLZF induces an intravascular surveillance program mediated by long-lived LFA-1-ICAM-1 interactions. *J Exp Med* **208**, 1179-88.

18 Lynch L, Michelet, X, Zhang, S, Brennan, PJ, Moseman, A, Lester, C, Besra, G, Vomhof-Dekrey, EE, Tighe, M, Koay, HF, Godfrey, DI, Leadbetter, EA, Sant'Angelo, DB, von Andrian, U & Brenner, MB (2015) Regulatory iNKT cells lack expression of the transcription factor PLZF and control the homeostasis of T(reg) cells and macrophages in adipose tissue. *Nat Immunol* **16**, 85-95.

19 Lee YJ, Holzapfel, KL, Zhu, J, Jameson, SC & Hogquist, KA (2013) Steady-state production of IL-4 modulates immunity in mouse strains and is determined by lineage diversity of iNKT cells. *Nat Immunol* **14**, 1146-54.

20 Lee YJ, Wang, H, Starrett, GJ, Phuong, V, Jameson, SC & Hogquist, KA (2015) Tissue-Specific Distribution of iNKT Cells Impacts Their Cytokine Response. *Immunity* **43**, 566-78.

21 Engel I, Seumois, G, Chavez, L, Samaniego-Castruita, D, White, B, Chawla, A, Mock, D, Vijayanand, P & Kronenberg, M (2016) Innate-like functions of natural killer T cell subsets result from highly divergent gene programs. *Nat Immunol* **17**, 728-39.

22 Sag D, Krause, P, Hedrick, CC, Kronenberg, M & Wingender, G (2014) IL-10-producing NKT10 cells are a distinct regulatory invariant NKT cell subset. *J Clin Invest* **124**, 3725-40.

23 Wang Y, Sedimbi, S, Lofbom, L, Singh, AK, Porcelli, SA & Cardell, SL (2018) Unique invariant natural killer T cells promote intestinal polyps by suppressing TH1 immunity and promoting regulatory T cells. *Mucosal Immunol* **11**, 131-143.

24 Chang PP, Barral, P, Fitch, J, Pratama, A, Ma, CS, Kallies, A, Hogan, JJ, Cerundolo, V, Tangye, SG, Bittman, R, Nutt, SL, Brink, R, Godfrey, DI, Batista, FD & Vinuesa, CG (2012) Identification of Bcl-6-dependent follicular helper NKT cells that provide cognate help for B cell responses. *Nat Immunol* **13**, 35-43.

25 Monteiro M, Almeida, CF, Caridade, M, Ribot, JC, Duarte, J, Agua-Doce, A, Wollenberg, I, Silva-Santos, B & Graca, L (2010) Identification of Regulatory Foxp3+ Invariant NKT Cells Induced by TGF- *J Immunol* **185**, 2157-2163.

26 Gaya M, Barral, P, Burbage, M, Aggarwal, S, Montaner, B, Warren Navia, A, Aid, M, Tsui, C, Maldonado, P, Nair, U, Ghneim, K, Fallon, PG, Sekaly, RP, Barouch, DH, Shalek, AK,

Bruckbauer, A, Strid, J & Batista, FD (2018) Initiation of Antiviral B Cell Immunity Relies on Innate Signals from Spatially Positioned NKT Cells. *Cell* **172**, 517-533 e20.

27 Tuttle KD, Krovi, SH, Zhang, J, Bedel, R, Harmacek, L, Peterson, LK, Dragone, LL, Lefferts, A, Halluszczak, C, Riemondy, K, Hesselberth, JR, Rao, A, O'Connor, BP, Marrack, P, Scott-Browne, J & Gapin, L (2018) TCR signal strength controls thymic differentiation of iNKT cell subsets. *Nature communications* **9**, 2650.

28 Zhao M, Svensson, MND, Venken, K, Chawla, A, Liang, S, Engel, I, Mydel, P, Day, J, Elewaut, D, Bottini, N & Kronenberg, M (2018) Altered thymic differentiation and modulation of arthritis by invariant NKT cells expressing mutant ZAP70. *Nature communications* **9**, 2627.

29 Uddin MN, Sultana, DA, Lorentsen, KJ, Cho, JJ, Kirst, ME, Brantly, ML, Califano, D, Sant'Angelo, DB & Avram, D (2016) Transcription factor Bcl11b sustains iNKT1 and iNKT2 cell programs, restricts iNKT17 cell program, and governs iNKT cell survival. *PNAS* **113**, 7608-13.

30 Drees C, Vahl, JC, Bortoluzzi, S, Heger, KD, Fischer, JC, Wunderlich, FT, Peschel, C & Schmidt-Supprian, M (2017) Roquin Paralogs Differentially Regulate Functional NKT Cell Subsets. *J Immunol* **198**, 2747-2759.

31 Monteiro M, Agua-Doce, A, Almeida, CF, Fonseca-Pereira, D, Veiga-Fernandes, H & Graca, L (2015) IL-9 Expression by Invariant NKT Cells Is Not Imprinted during Thymic Development. *J Immunol* **195**, 3463-71.

32 Monteiro M, Almeida, CF, Agua-Doce, A & Graca, L (2013) Induced IL-17-producing invariant NKT cells require activation in presence of TGF-beta and IL-1beta. *J Immunol* **190**, 805-11.

33 Wang H & Hogquist, KA (2018) CCR7 defines a precursor for murine iNKT cells in thymus and periphery. *Elife* **7**.

34 Milpied P, Massot, B, Renand, A, Diem, S, Herbelin, A, Leite-de-Moraes, M, Rubio, MT & Hermine, O (2011) IL-17-producing invariant NKT cells in lymphoid organs are recent thymic emigrants identified by neuropilin-1 expression. *Blood* **118**, 2993-3002.

35 Saez de Guinoa J, Jimeno, R, Gaya, M, Kipling, D, Garzon, MJ, Dunn-Walters, D, Ubeda, C & Barral, P (2018) CD1d-mediated lipid presentation by CD11c(+) cells regulates intestinal homeostasis. *Embo J* **37**.

36 Brennan PJ, Brigl, M & Brenner, MB (2013) Invariant natural killer T cells: an innate activation scheme linked to diverse effector functions. *Nat Rev Immunol* **13**, 101-17.

37 Kinjo Y, Tupin, E, Wu, D, Fujio, M, Garcia-Navarro, R, Benhnia, MR, Zajonc, DM, Ben-Menachem, G, Ainge, GD, Painter, GF, Khurana, A, Hoebe, K, Behar, SM, Beutler, B, Wilson, IA, Tsuji, M, Sellati, TJ, Wong, CH & Kronenberg, M (2006) Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nat Immunol* **7**, 978-86.

38 Wieland Brown LC, Penaranda, C, Kashyap, PC, Williams, BB, Clardy, J, Kronenberg, M, Sonnenburg, JL, Comstock, LE, Bluestone, JA & Fischbach, MA (2013) Production of alpha-galactosylceramide by a prominent member of the human gut microbiota. *PLoS Biol* **11**, e1001610.

39 Albacker LA, Chaudhary, V, Chang, YJ, Kim, HY, Chuang, YT, Pichavant, M, DeKruyff, RH, Savage, PB & Umetsu, DT (2013) Invariant natural killer T cells recognize a fungal glycosphingolipid that can induce airway hyperreactivity. *Nat Med* **19**, 1297-304.

40 Abos Gracia B, Lopez Relano, J, Revilla, A, Castro, L, Villalba, M, Martin Adrados, B, Regueiro, JR, Fernandez-Malave, E, Martinez Naves, E & Gomez Del Moral, M (2017) Human Invariant Natural Killer T Cells Respond to Antigen-Presenting Cells Exposed to Lipids from Olea europaea Pollen. *Int Arch Allergy Immunol* **173**, 12-22.

41 Shissler SC, Lee, MS & Webb, TJ (2017) Mixed Signals: Co-Stimulation in Invariant Natural Killer T Cell-Mediated Cancer Immunotherapy. *Frontiers in immunology* **8**, 1447.

42 Gapin L, Godfrey, DI & Rossjohn, J (2013) Natural Killer T cell obsession with self-antigens. *Curr Opin Immunol* **25**, 168-73.

43 Paget C, Mallevaey, T, Speak, AO, Torres, D, Fontaine, J, Sheehan, KC, Capron, M, Ryffel, B, Faveeuw, C, Leite de Moraes, M, Platt, F & Trottein, F (2007) Activation of invariant NKT cells by toll-like receptor 9-stimulated dendritic cells requires type I interferon and charged glycosphingolipids. *Immunity* **27**, 597-609.

44 Darmoise A, Teneberg, S, Bouzonville, L, Brady, RO, Beck, M, Kaufmann, SH & Winau, F (2010) Lysosomal alpha-galactosidase controls the generation of self lipid antigens for natural killer T cells. *Immunity* **33**, 216-28.

45 Paget C, Deng, S, Soulard, D, Priestman, DA, Speca, S, von Gerichten, J, Speak, AO, Saroha, A, Pewzner-Jung, Y, Futerman, AH, Mallevaey, T, Faveeuw, C, Gu, X, Platt, FM, Sandhoff, R & Trottein, F (2019) TLR9-mediated dendritic cell activation uncovers mammalian ganglioside species with specific ceramide backbones that activate invariant natural killer T cells. *PLoS Biol* **17**, e3000169.

46 Velázquez P, Cameron, TO, Kinjo, Y, Nagarajan, N, Kronenberg, M & Dustin, ML (2008) Cutting edge: activation by innate cytokines or microbial antigens can cause arrest of natural killer T cell patrolling of liver sinusoids. *J Immunol* **180**, 2024-8.

47 Holzapfel KL, Tyznik, AJ, Kronenberg, M & Hogquist, KA (2014) Antigen-dependent versus -independent activation of invariant NKT cells during infection. *J Immunol* **192**, 5490-8.

48 Brigl M, Tatituri, RV, Watts, GF, Bhowruth, V, Leadbetter, EA, Barton, N, Cohen, NR, Hsu, FF, Besra, GS & Brenner, MB (2011) Innate and cytokine-driven signals, rather than microbial

antigens, dominate in natural killer T cell activation during microbial infection. *J Exp Med* **208**, 1163-77.

49 Loh L, Ivarsson, MA, Michaelsson, J, Sandberg, JK & Nixon, DF (2014) Invariant natural killer T cells developing in the human fetus accumulate and mature in the small intestine. *Mucosal Immunol* **7**, 1233-43.

50 Olszak T, An, D, Zeissig, S, Vera, MP, Richter, J, Franke, A, Glickman, JN, Siebert, R, Baron, RM, Kasper, DL & Blumberg, RS (2012) Microbial Exposure During Early Life Has Persistent Effects on Natural Killer T Cell Function. *Science* **336**, 489-493.

51 An D, Oh, SF, Olszak, T, Neves, JF, Avci, FY, Erturk-Hasdemir, D, Lu, X, Zeissig, S, Blumberg, RS & Kasper, DL (2014) Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells. *Cell* **156**, 123-33.

52 Wingender G, Stepniak, D, Krebs, P, Lin, L, McBride, S, Wei, B, Braun, J, Mazmanian, SK & Kronenberg, M (2012) Intestinal microbes affect phenotypes and functions of invariant natural killer T cells in mice. *Gastroenterol* **143**, 418-28.

53 Clancy-Thompson E, Chen, GZ, LaMarche, NM, Ali, LR, Jeong, HJ, Crowley, SJ, Boelaars, K, Brenner, MB, Lynch, L & Dougan, SK (2019) Transnuclear mice reveal Peyer's patch iNKT cells that regulate B-cell class switching to IgG1. *Embo J* **38**, e101260.

54 Wei B, Wingender, G, Fujiwara, D, Chen, DY, Mcpherson, M, Brewer, S, Borneman, J, Kronenberg, M & Braun, J (2010) Commensal Microbiota and CD8+ T Cells Shape the Formation of Invariant NKT Cells. *J Immunol* **184**, 1218-1226.

55 Moran AE, Holzapfel, KL, Xing, Y, Cunningham, NR, Maltzman, JS, Punt, J & Hogquist, KA (2011) T cell receptor signal strength in Treg and iNKT cell development demonstrated by a novel fluorescent reporter mouse. *J Exp Med* **208**, 1279-89.

56 Burrello C, Garavaglia, F, Cribiu, FM, Ercoli, G, Bosari, S, Caprioli, F & Facciotti, F (2018) Short-term Oral Antibiotics Treatment Promotes Inflammatory Activation of Colonic Invariant Natural Killer T and Conventional CD4(+) T Cells. *Front Med (Lausanne)* **5**, 21.

57 Ma C, Han, M, Heinrich, B, Fu, Q, Zhang, Q, Sandhu, M, Agdashian, D, Terabe, M, Berzofsky, JA, Fako, V, Ritz, T, Longerich, T, Theriot, CM, McCulloch, JA, Roy, S, Yuan, W, Thovarai, V, Sen, SK, Ruchirawat, M, Korangy, F, Wang, XW, Trinchieri, G & Greten, TF (2018) Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* **360**.

58 Kinjo Y, Wu, D, Kim, G, Xing, GW, Poles, MA, Ho, DD, Tsuji, M, Kawahara, K, Wong, CH & Kronenberg, M (2005) Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* **434**, 520-5.

59 Sriram V, Du, W, Gervay-Hague, J & Brutkiewicz, RR (2005) Cell wall glycosphingolipids of Sphingomonas paucimobilis are CD1d-specific ligands for NKT cells. *Eur J Immunol* **35**, 1692-701.

60 von Gerichten J, Lamprecht, D, Opalka, L, Soulard, D, Marsching, C, Pilz, R, Sencio, V, Herzer, S, Galy, B, Nordstrom, V, Hopf, C, Grone, HJ, Trottein, F & Sandhoff, R (2019) Bacterial immunogenic alpha-galactosylceramide identified in the murine large intestine: dependency on diet and inflammation. *J Lipid Res*.

61 Bai L, Constantinides, MG, Thomas, SY, Reboulet, R, Meng, F, Koentgen, F, Teyton, L, Savage, PB & Bendelac, A (2012) Distinct APCs explain the cytokine bias of alpha-galactosylceramide variants in vivo. *J Immunol* **188**, 3053-61.

62 Hepworth MR, Monticelli, LA, Fung, TC, Ziegler, CG, Grunberg, S, Sinha, R, Mantegazza, AR, Ma, HL, Crawford, A, Angelosanto, JM, Wherry, EJ, Koni, PA, Bushman, FD, Elson, CO, Eberl, G, Artis, D & Sonnenberg, GF (2013) Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. *Nature* **498**, 113-7.

63 Hepworth MR, Fung, TC, Masur, SH, Kelsen, JR, McConnell, FM, Dubrot, J, Withers, DR, Hugues, S, Farrar, MA, Reith, W, Eberl, G, Baldassano, RN, Laufer, TM, Elson, CO & Sonnenberg, GF (2015) Immune tolerance. Group 3 innate lymphoid cells mediate intestinal selection of commensal bacteria-specific CD4(+) T cells. *Science* **348**, 1031-5.

64 Saez de Guinoa J, Jimeno, R, Farhadi, N, Jervis, PJ, Cox, LR, Besra, GS & Barral, P (2017) CD1d-mediated activation of group 3 innate lymphoid cells drives IL-22 production. *Embo Rep* **18**, 39-47.

65 Nieuwenhuis EES, Matsumoto, T, Lindenbergh, D, Willemsen, R, Kaser, A, Simons-Oosterhuis, Y, Brugman, S, Yamaguchi, K, Ishikawa, H, Aiba, Y, Koga, Y, Samsom, JN, Oshima, K, Kikuchi, M, Escher, JC, Hattori, M, Onderdonk, AB & Blumberg, RS (2009) Cd1d-dependent regulation of bacterial colonization in the intestine of mice. *J Clin Invest* **119**, 1241-1250.

66 Farin HF, Karthaus, WR, Kujala, P, Rakhshandehroo, M, Schwank, G, Vries, RG, Kalkhoven, E, Nieuwenhuis, EE & Clevers, H (2014) Paneth cell extrusion and release of antimicrobial products is directly controlled by immune cell-derived IFN-gamma. *J Exp Med* **211**, 1393-405.

67 Barral P, Eckl-Dorna, J, Harwood, NE, De Santo, C, Salio, M, Illarionov, P, Besra, GS, Cerundolo, V & Batista, FD (2008) B cell receptor-mediated uptake of CD1d-restricted antigen augments antibody responses by recruiting invariant NKT cell help in vivo. *PNAS* **105**, 8345-50.

68 Leadbetter EA, Brigl, M, Illarionov, P, Cohen, N, Luteran, MC, Pillai, S, Besra, GS & Brenner, MB (2008) NK T cells provide lipid antigen-specific cognate help for B cells. *PNAS* **105**, 8339-44.

69 King IL, Fortier, A, Tighe, M, Dibble, J, Watts, GFM, Veerapen, N, Haberman, AM, Besra, GS, Mohrs, M, Brenner, MB & Leadbetter, EA (2012) Invariant natural killer T cells direct B cell responses to cognate lipid antigen in an IL-21-dependent manner. *Nat Immunol* **13**, 44-50.

70 Cerutti A & Rescigno, M (2008) The biology of intestinal immunoglobulin A responses. *Immunity* **28**, 740-50.

71 Koch MA, Reiner, GL, Lugo, KA, Kreuk, LS, Stanbery, AG, Ansaldo, E, Seher, TD, Ludington, WB & Barton, GM (2016) Maternal IgG and IgA Antibodies Dampen Mucosal T Helper Cell Responses in Early Life. *Cell* **165**, 827-41.

72 Barnes MJ & Powrie, F (2009) Regulatory T cells reinforce intestinal homeostasis. *Immunity* **31**, 401-11.

73 Eidenschenk C, Rutz, S, Liesenfeld, O & Ouyang, W (2014) Role of IL-22 in microbial host defense. *Current topics in microbiology and immunology* **380**, 213-36.

74 Olszak T, Neves, JF, Dowds, CM, Baker, K, Glickman, J, Davidson, NO, Lin, CS, Jobin, C, Brand, S, Sotlar, K, Wada, K, Katayama, K, Nakajima, A, Mizuguchi, H, Kawasaki, K, Nagata, K, Muller, W, Snapper, SB, Schreiber, S, Kaser, A, Zeissig, S & Blumberg, RS (2014) Protective mucosal immunity mediated by epithelial CD1d and IL-10. *Nature* **509**, 497-502.

75 Xavier RJ & Podolsky, DK (2007) Unravelling the pathogenesis of inflammatory bowel disease. *Nature* **448**, 427-34.

76 Heller F, Fuss, IJ, Nieuwenhuis, EE, Blumberg, RS & Strober, W (2002) Oxazolone colitis, a Th2 colitis model resembling ulcerative colitis, is mediated by IL-13-producing NK-T cells. *Immunity* **17**, 629-38.

77 Kim HS & Chung, DH (2013) IL-9-producing invariant NKT cells protect against DSS-induced colitis in an IL-4-dependent manner. *Mucosal Immunol* **6**, 347-57.

78 Weigmann B, Lehr, HA, Yancopoulos, G, Valenzuela, D, Murphy, A, Stevens, S, Schmidt, J, Galle, PR, Rose-John, S & Neurath, MF (2008) The transcription factor NFATc2 controls IL-6-dependent T cell activation in experimental colitis. *J Exp Med* **205**, 2099-110.

79 Camelo A, Barlow, JL, Drynan, LF, Neill, DR, Ballantyne, SJ, Wong, SH, Pannell, R, Gao, W, Wrigley, K, Sprenkle, J & McKenzie, AN (2012) Blocking IL-25 signalling protects against gut inflammation in a type-2 model of colitis by suppressing nuocyte and NKT derived IL-13. *J Gastroenterol* **47**, 1198-211.

80 Fuss IJ, Heller, F, Boirivant, M, Leon, F, Yoshida, M, Fitchtner-Feigl, S, Yang, Z, Exley, MA, Kitani, A, Blumberg, RS, Mannon, P & Strober, W (2004) Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J Clin Invest* **113**, 1490-1497.

81 Fuss IJ, Joshi, B, Yang, Z, Degheidy, H, Fichtner-Feigl, S, de Souza, H, Rieder, F, Scaldaferri, F, Schirbel, A, Scarpa, M, West, G, Yi, C, Xu, L, Leland, P, Yao, M, Mannon, P, Puri, RK, Fiocchi, C & Strober, W (2014) IL-13Ralpha2-bearing, type II NKT cells reactive to sulfatide self-antigen populate the mucosa of ulcerative colitis. *Gut* **63**, 1728-36.

82 Heller F, Fromm, A, Gitter, AH, Mankertz, J & Schulzke, JD (2008) Epithelial apoptosis is a prominent feature of the epithelial barrier disturbance in intestinal inflammation: effect of pro-inflammatory interleukin-13 on epithelial cell function. *Mucosal Immunol* **1 Suppl 1**, S58-61.

83 Heller F, Florian, P, Bojarski, C, Richter, J, Christ, M, Hillenbrand, B, Mankertz, J, Gitter, AH, Burgel, N, Fromm, M, Zeitz, M, Fuss, I, Strober, W & Schulzke, JD (2005) Interleukin-13 is the key effector Th2 cytokine in ulcerative colitis that affects epithelial tight junctions, apoptosis, and cell restitution. *Gastroenterol* **129**, 550-64.

84 Danese S, Rudzinski, J, Brandt, W, Dupas, JL, Peyrin-Biroulet, L, Bouhnik, Y, Kleczkowski, D, Uebel, P, Lukas, M, Knutsson, M, Erlandsson, F, Hansen, MB & Keshav, S (2015) Tralokinumab for moderate-to-severe UC: a randomised, double-blind, placebo-controlled, phase IIa study. *Gut* **64**, 243-9.

85 Hoving JC (2018) Targeting IL-13 as a Host-Directed Therapy Against Ulcerative Colitis. *Front Cell Infect Microbiol* **8**, 395.

86 O'Brien TF, Bao, K, Dell'Aringa, M, Ang, WX, Abraham, S & Reinhardt, RL (2016) Cytokine expression by invariant natural killer T cells is tightly regulated throughout development and settings of type-2 inflammation. *Mucosal Immunol* **9**, 597-609.

87 Iyer SS, Gensollen, T, Gandhi, A, Oh, SF, Neves, JF, Collin, F, Lavin, R, Serra, C, Glickman, J, de Silva, PSA, Sartor, RB, Besra, G, Hauser, R, Maxwell, A, Llebaria, A & Blumberg, RS (2018) Dietary and Microbial Oxazoles Induce Intestinal Inflammation by Modulating Aryl Hydrocarbon Receptor Responses. *Cell* **173**, 1123-1134 e11.

88 Liao CM, Zimmer, MI, Shanmuganad, S, Yu, HT, Cardell, SL & Wang, CR (2012) dysregulation of CD1d-restricted type ii natural killer T cells leads to spontaneous development of colitis in mice. *Gastroenterol* **142**, 326-34 e1-2.

89 Burrello C, Pellegrino, G, Giuffre, MR, Lovati, G, Magagna, I, Bertocchi, A, Cribiu, FM, Boggio, F, Botti, F, Trombetta, E, Porretti, L, Di Sabatino, A, Vecchi, M, Rescigno, M, Caprioli, F & Facciotti, F (2019) Mucosa-associated microbiota drives pathogenic functions in IBD-derived intestinal iNKT cells. *Life Sci Alliance* **2**.

90 Saubermann LJ, Beck, P, De Jong, YP, Pitman, RS, Ryan, MS, Kim, HS, Exley, M, Snapper, S, Balk, SP, Hagen, SJ, Kanauchi, O, Motoki, K, Sakai, T, Terhorst, C, Koezuka, Y, Podolsky, DK & Blumberg, RS (2000) Activation of natural killer T cells by alpha-galactosylceramide in the presence of CD1d provides protection against colitis in mice. *Gastroenterol* **119**, 119-28.

91 Lee SW, Park, HJ, Cheon, JH, Wu, L, Van Kaer, L & Hong, S (2018) iNKT Cells Suppress Pathogenic NK1.1(+)CD8(+) T Cells in DSS-Induced Colitis. *Frontiers in immunology* **9**, 2168.

92 Selvanantham T, Lin, Q, Guo, CX, Surendra, A, Fieve, S, Escalante, NK, Guttman, DS, Streutker, CJ, Robertson, SJ, Philpott, DJ & Mallevaey, T (2016) NKT Cell-Deficient Mice Harbor an Altered Microbiota That Fuels Intestinal Inflammation during Chemically Induced Colitis. *J Immunol* **197**, 4464-4472.

93 Shen S, Prame Kumar, K, Stanley, D, Moore, RJ, Van, TTH, Wen, SW, Hickey, MJ & Wong, CHY (2018) Invariant Natural Killer T Cells Shape the Gut Microbiota and Regulate Neutrophil Recruitment and Function During Intestinal Inflammation. *Frontiers in immunology* **9**, 999.

Table 1. Properties and tissue distribution of iNKT cell subsets. Characteristic surface markers, transcription factors, tissue distribution and cytokine production for iNKT subsets in C57Bl/6 mice. Cytokines shown in bold are the prototypical cytokines secreted by each iNKT cell subset (iLN=inguinal LN, aLN= axillary LN, cLN= cervical LN; n.r.= not reported; n.d = not detected)

iNKT subset	Transcription factors	Surface Markers	Cytokines	Tissues (C57Bl/6 steady-state)	References
NKT1	T-bet, $PLZF^{low}$, GATA3	$CD4^{+/-}$, $NK1.1^{+}$, $CXCR3^{+}$, IL-$17RB^{-}$	**IFNγ** TNFα, IL-4, granzyme,perforin	Enriched in liver, spleen, thymus	[19-21]
NKT2	$PLZF^{High}$, GATA3	$CD4^{+}$, $NK1.1^{-}$, $CD27^{+}$, IL-$25R^{+}$	**IL-4, IL-13** IL-6	Enriched in mLN	[19-21]
NKT17	RORγt, $PLZF^{int}$, GATA-3,	$CD4^{-}$, $NK1.1^{-}$, $CD127^{High}$, IL-$23R^{+}$, $CCR6^{+}$, $CD103^{+}$	**IL-17** IL-22	Enriched in lung, iLN, aLN, cLN	[19-21]
NKT10	$PLZF^{Low}$, E4BP4	$PD1^{+}$, $Nrp1^{+}$, $CD49d^{+}$	**IL-10** IL-2	Fat	[18]
NKTreg	$Foxp3^{+}$	$NK1.1^{-}$, $CD25^{+}$, $GITR^{+}$, $NKG2D^{+}$	**n.r.**	n.d	[24]
NKTFH	BCL6	$CXCR5^{+}$, $PD1^{+}$	**IL-21**	n.d	[25]

FIGURE LEGENDS

Figure 1. Mechanisms of iNKT cell activation. (A) Direct activation via microbial or synthetic lipids: Microbial lipid antigens from bacteria (such as *Sphingomonas* or *Borrelia*) or synthetic lipids (such as αGalCer) will be loaded in CD1d and directly recognised by the T cell receptor (TCR) of iNKT cells inducing their activation and cytokine secretion [37, 58]. iNKT cell activation in this context can be further modulated by cytokines and/or co-stimulation [41]. (B) Endogenous lipids + innate signals: Toll-like receptor (TLR) stimulation of antigen presenting cells (APCs) induces changes in lipid synthesis and presentation of endogenous lipids. TLR-stimulated APCs secrete IL-12 that together with endogenous lipids mediate iNKT cell activation [8, 44, 45]. (C) Cytokine mediated activation: Several cytokines such as IL-12, IL-18 or type I IFN are sufficient to induce iNKT activation in a CD1d-independent manner [46, 47].

Figure 2. NKT cells in the regulation of intestinal homeostasis. iNKT cells contribute to the communication between the microbiota and the intestinal immune system. Intestinal iNKT cells sense lipids presented by $CD11c^+$ cells and this regulates iNKT cell homeostasis and activation [35]. In turn, iNKT cells modulate (both directly and indirectly) the function of other intestinal immune cells as well as the composition and stratification of intestinal bacteria [35, 65]. CD1d-mediated crosstalk between iNKT cells and intestinal epithelial cells (IEC) modulates IL-10 secretion [74], while engagement of CD1d on group 3 innate lymphoid cells (ILC3) induces IL-22 production [64]; both of these cytokines contribute to the control of intestinal homeostasis. Moreover, iNKT cells indirectly regulate the intestinal Treg population [35], the secretion of IgG1 by intestinal B cells [53] and Paneth cell (PC) degranulation [65, 66].

Figure 3. NKT cells in intestinal inflammation. In human ulcerative colitis or murine models of oxazolone colitis NKT cells contribute to the fueling of intestinal inflammation through a

mechanism mediated by IL-13 [74, 76, 79-81]. IL-13 can induce apoptosis of intestinal epithelial cells (IEC) and alter epithelial tight junctions which in turn will impair barrier function [82, 83].

ACKNOWLEDGMENTS

This work was funded by the UK's Medical Research Council and the Biotechnology and Biological Sciences Research Council (grants to P.B. MR/L008157/1 and BB/S005560/1); P.M.B. was supported by a studentship from the Medical Research Council and King's College London Doctoral Training Partnership in Biomedical Sciences (MR/N013700/1). M.L-F is funded by a Francis Crick Institute-King's College London studentship.

AUTHORS CONTRIBUTION

All authors wrote the manuscript

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Figure 1

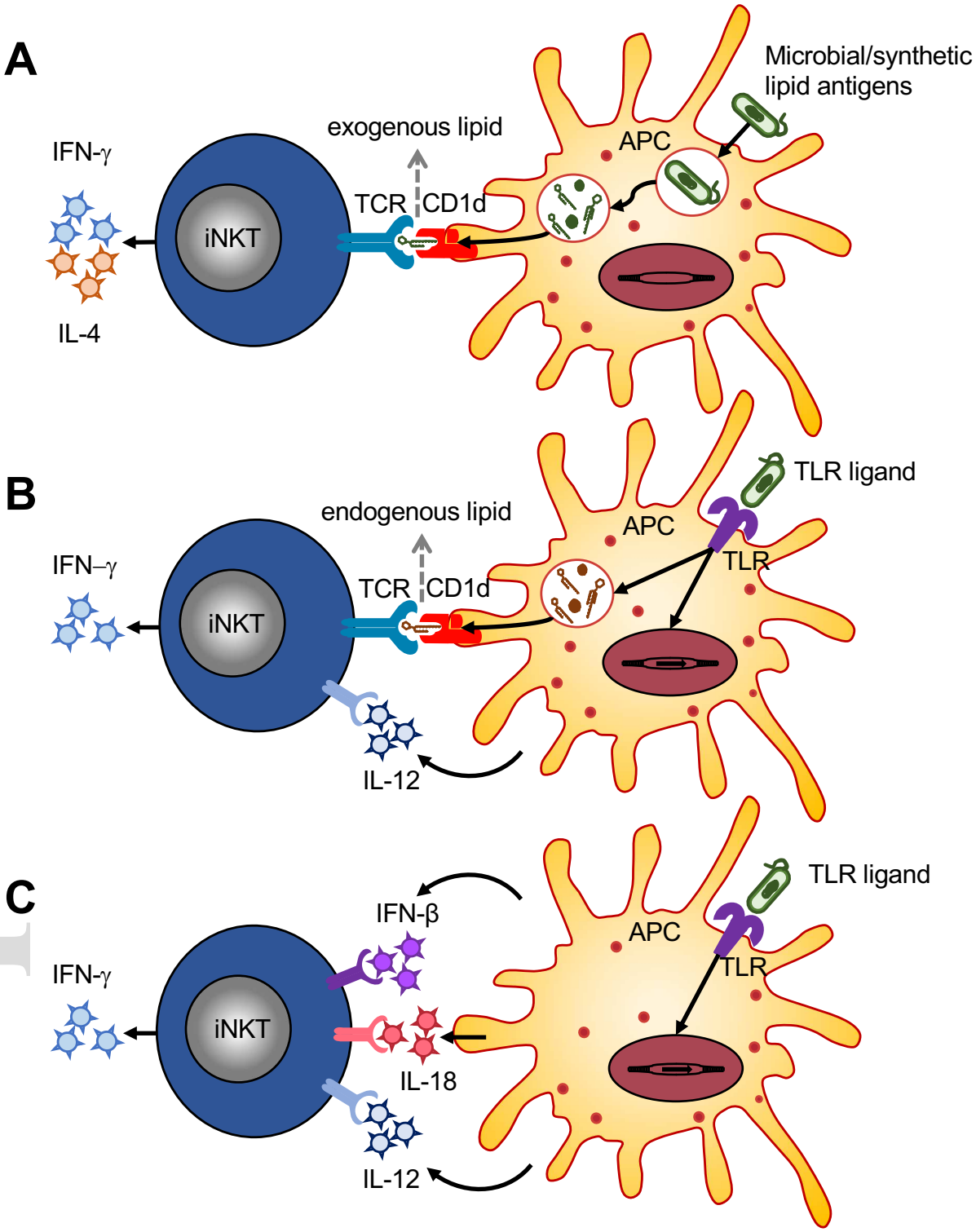

FIGURE 2

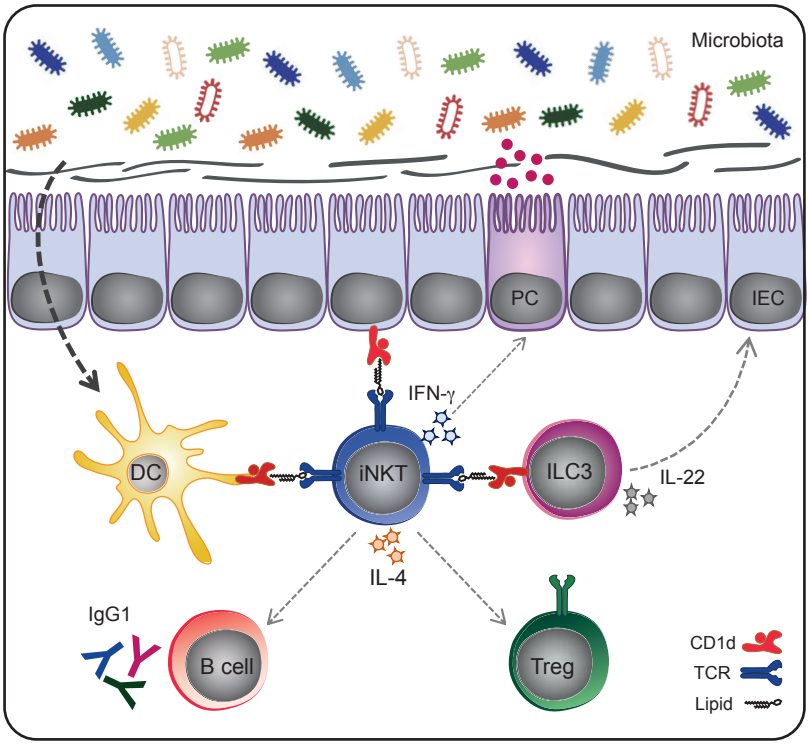

FIGURE 3